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OBSERVATIONS ON THE DISTRIBUTION AND LIFE HISTORY OF *CEPHALOBIUM MICROBIVORUM* COBB AND OF ITS HOST, *GRYLLUS ASSIMILIS* FABRICIUS[1]

By

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Contents

[1] Contribution No. 52 from the Zoology Department, Agricultural Experiment Station of the Kansas State Agricultural College.

Introduction

While securing gregarines from black field crickets for class use, in October, 1918, the senior writer found heavy infestations of small nematodes, some of which were sent to Dr. N. A. Cobb for identification. Determining that these nematodes represent a new genus, Doctor Cobb suggested that studies be made on the distribution and life history of it. Observations on its distribution have been made at Woods Hole, Mass.; Falls Church, Va.; Douglas Lake, Mich.; Rockford, Ill.; and Manhattan, Kan. The studies on its life history and that of the crickets were made at Manhattan. Further work on certain phases of these studies would be very desirable, but as this cannot be done for some time, it seems best to put the present findings on record. The writers wish to express their indebtedness to Doctor Cobb for suggesting this nematode study, and to Director Frank R. Lillie of the Marine Biological Laboratory, Woods Hole, Mass., and Director George R. LaRue of the University of Michigan Biological Station for the privilege of using equipment at the respective stations.

The Parasite, Cephalobium microbivorum Cobb

Description

These nematodes which are from 2 to 3 mm. in length were identified as *Cephalobium microbivorum* n. g., n. sp., by Dr. N. A. Cobb, who submits this description.

The following characterizations and description, with figures, are taken from "Contribution to a Science of Nematology," No. IX; *"One Hundred New Nemas,"* N. A. Cobb, 1920. The characters other than specific are assembled from Cobb's Keys.

Phylum Nemates

Subphylum Laimia: Nemas having a more or less distinct pharynx.

Class Anonchia: Nemas lacking onchia.

Subclass Anodontia: Nemas lacking odontia.

Order Polylaimia: Nemas having an unarmed pharynx, composed of two or more successive chambers more or less distinctly separated from each other.

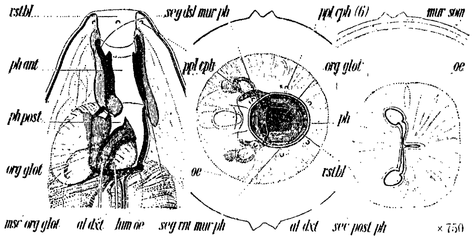
Genus Cephalobium

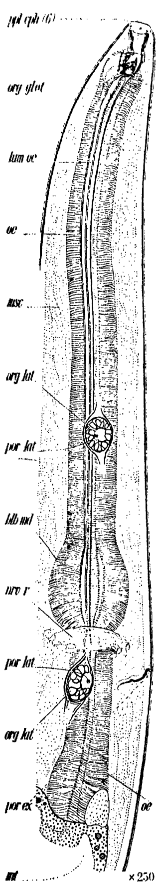
Cavity of the pharynx more or less prismoid or cylindroid (not conoid or very irregular), and containing a glottoid organ at its base. Oesophagus with median bulb and posterior swelling. Amphids none so far as known. Seta-like labial papillae 6. Single lateral wing present; striae fine, plain. Spinneret absent.

Preanal and postanal papillae present on the male. Tail conoid or subconoid; terminus acute, unarmed. Bursa none. Spicula two, equal, more or less arcuate; not jointed; their width not uniform. Accessories (gubernaculum) present. Inner ends of spicula cephalated by constriction. Length of the spicula 1¼ times as great as anal body diameter.

54. *Cephalobium microbivorum*, n. sp. The single wing begins near the head and ends near the terminus. Its optical expression is either a pair of lines or a single line in the middle of a field one-twelfth as wide as the body. The contour of the body may become crenate in the anal region. There are about thirteen lateral organs on each side connected with pores

in the cuticle (see org. lat. fig. *54*). Base of the pharynx containing a large, complicated and peculiar dorsal glottoid organ (see fig. *54*). No amphids. The rather thin-walled intestine is set off by a collum one-eighth as wide as the neck, and has a rather distinct lumen. It becomes at once five-sixths as wide as the body, and in cross section presents two to four cells. From the somewhat depressed anus, the narrow, cutinized rectum extends inward and forward a distance one and one-fourth times as great as the anal body-diameter. Scattered yellowish granules of variable size occur in the cells of the intestine, the largest being one twenty-fifth as wide as the body; in addition, there are numerous very small granules. There is no tessellated effect. Subarcuate, conoid tail tapers from in front of the anus to the acute, fine terminus. There is no spinneret. From the elevated vulva, the rather small, somewhat weak vagina extends inward nearly at right angles to the ventral surface one-fourth the way across the body. Along the middle half of the body the two equal uteri contain thin-shelled, smooth, ellipsoidal eggs two-thirds as long as the body is wide, and appear to be deposited after segmentation begins. No embryos were seen in these eggs, only blastulas. For the most part the ova are arranged irregularly in the somewhat tapering ovaries. The two equal, rather strong, slender, arcuate, tapering subacute spicula are one and one-fourth times as long as the anal body-diameter, are more or less cephalated by constriction and when seen in profile have proximal ends nearly opposite the body-axis. Toward their distal ends four slender stiffening pieces are apparent. There is a simple, strong, and rather solid, straight accessory piece, one-third as long as the spicula, bending back from them at an angle of about 90 degrees, so that its proximal end lies opposite the body-diameter. There is no bursa. Near the beginning of the second quarter of the tail there is a pair of lateral pores similar to those on the female. Beginning just in front of the anus there exists on the tail a series of six submedian pairs of flattish-conoid, rather inconspicuous papillae. These occupy the anterior two-thirds of the tail, and have a formula as follows: 1 ( ) 1; 111; 1. The members of the posterior four pairs are located exactly opposite each other, the right hand member of each pair being slightly behind the left hand member. Papillae plainly enervated. Spicula conspicuous, rather close together; at the widest part about one-eighth as wide as the corresponding portion of the body, ending in minute "buttons." The lateral pores on the tail are the final members of the lateral series of lateral organs.





1.	10.3	12.8	31 '48' 30	86.	3. mm
1.	2.5	2.6	3.3	1.6	
1.2	10.	13.	74 M	90.	2.8 mm
1.1	2.4	2.8	2.9	1.9	

Habitat: Intestine of the field cricket, *Gryllus assimilis* Fabr. The males are considerably smaller than the adult gravid females.

HABITAT

To ascertain the part of the cricket inhabited by *C. microbivorum* some crickets were carefully dissected. In each case the thin-walled ileum readily revealed the writhing nematodes which appeared in bold contrast to the dark fecal contents. None of these parasites was found in the coelom, nor in any organ outside of the digestive tract. In one case two dead adults were taken from the colon of a freshly dissected cricket, whose ileum contained several live specimens. To facilitate subsequent discussion a brief description and a diagram are given of the alimentary canal of the cricket host.

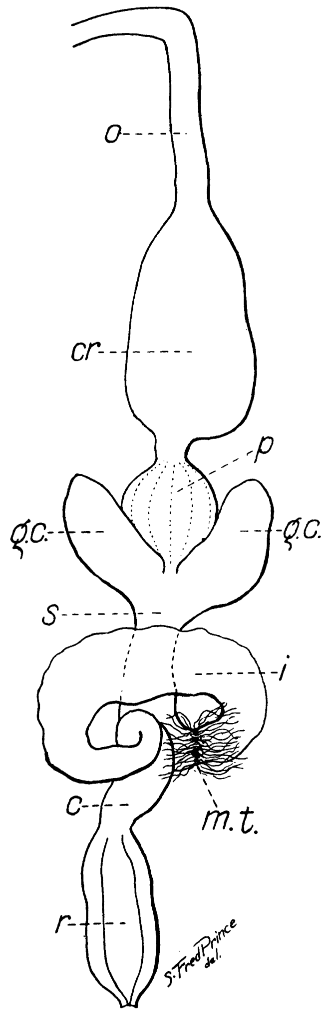


Fig. 1

Figure 1. Showing digestive tract of *Gryllus assimilis*. *c*, colon; *cr*, crop; *g.c.*, gastric caecum; *i*, ileum; *m.t.*, malpighian tubes; *o*, oesophagus; *p*, proventriculus; *r*, rectum; *s*, stomach. × 4.

The digestive tract (fig. 1) of this black field cricket, *Gryllus assimilis*, bears many resemblances to that of the large and nearly wingless western cricket, *Anabrus*, as shown by Packard (1878-79, p. 175). The oesophagus (o) connects with the mouth and after making a sharp bend proceeds through the posterior part of the head to open into the spacious crop (cr) which occupies the thoracic and anterior abdominal portion of the coelom. After greatly narrowing, the crop opens into the strong proventriculus (p) which is much larger than the corresponding organ in *Anabrus*. In *G. assimilis* the diameter of this organ exceeds three times that of the junction with the crop, whereas in *Anabrus* the proventriculus is considerably reduced in size.

The proventriculus opens by a very narrow canal into the true stomach (s) which immediately gives off anteriorly two large, flattened gastric caeca (g.c.), situated one above the other. The stomach is surprisingly slender. After passing backward a short distance it makes an abrupt turn upward, narrows slightly and terminates, giving off numerous Malpighian tubes (m. t.) where it joins the intestine. Like *Anabrus* the remainder of the digestive tract is distinctly divisible into three portions: the ileum, colon, and rectum. Unlike the large western species, however, the stomach of *G. assimilis* is considerably shorter and the ileum (i) longer and much more capacious. The walls of the stomach are thick and translucent, while those of the ileum are thin and transparent. So thin are the walls of the latter that not only are the enclosed, motile nematodes visible, but also the eggs in the females' bodies. Before terminating, the ileum narrows considerably, makes a pronounced twist and then opens into the larger, thick-walled colon (c). Continuing posteriorly, the colon with a slight constriction connects with the still larger rectum (r) with which the anus communicates.

Methods of Procedure

REMOVAL OF NEMATODES

The principal method employed for the removal of the nematodes is here briefly described. After excising the head of the cricket an incision was made in the posteroventral wall of the abdomen with fine scissors. By cutting forward through the median ventral surface, being careful not to cut deeply, nearly the whole lower body wall could be laid open without disturbing the digestive tract. On inserting fine forceps into the mesothoracic region the crop could be seized, and with slow, sustained effort the entire digestive tract withdrawn. In this extended condition the food tube was placed upon an ordinary glass slide, the rectum being excised at the anus. After covering with a few drops of normal salt solution, the intestine was teased with needles. The optical examinations were made with the aid of dissecting, binocular, and compound microscopes.

CULTURING OF NEMATODES

The culturing of the nematodes and their eggs was carried on for a time with fair success, but this phase of the problem should be continued. Having the nematodes on regular microscopic slides made it easy to remove the substances not wanted in the culture, and to add materials desired. With a little care the culture could be held to a restricted area on the slide by the surface tension of the solution. To prevent drying, the culture slides were placed in Petri dishes containing a few drops of distilled water. Subsequent microscopic examinations of the cultures were made either in or out of these small moist chambers.

Culture fluids used included normal salt solution alone, with fecal material, with peptone, and with both fecal material and peptone. Eggs hatched in each fluid, but growth of young nematodes appeared to occur only when a few drops of 0.8% peptone (in distilled water) were added. Dilute peptone was one of the successful solutions used by Welch and Wehrle (1918, p. 151) in their extensive nematode cultures.

In normal saline solution adult nematodes lived from one to six days, eggs developed and hatched, but even vigorous free embryos failed to increase in length and died within three days. When a few drops of 0.8% peptone were added to normal saline, embryos hatched and lived six days. The best results were obtained with equal volumes of normal saline and 0.8% peptone solution and a trace of cricket feces. In this medium several free embryos lived eight days, and a few thirteen days, the latter increasing their body lengths 16⅔ per cent. However, before the young nematodes had developed markedly, the culturing had to be abandoned on account of failure to secure nematodes. The adult crickets which had been collected prior to a cold wave succumbed in a few days, thus destroying the source of supply.

Observations on Development

Early cleavage to coiled embryo

The observations on development were upon living material, no attempt being made to trace the formation of germ layers or organs. The translucency of the dividing cells made it possible to follow the individual blastomeres until they formed a more or less spherical mass, such as shown in fig. 6. Whether such developing forms as represented in figs. 6 and 7 were hollow is uncertain, owing to the growing opacity of the embryos. For convenience in description the terminology of Martin (1913) is partly followed. For the uncertain stages shown in figs. 6 and 7, *a* morula rather than *the* morula is used. Likewise, when the vermiform shape of the embryo is first attained (figs. 8, 9) the term *curved* embryos is employed, whereas, the fully attenuated enclosed embryo (fig. 11) is designated as a *coiled* embryo.

These few observations on the development of the external form of *C. microbivorum* are given in the hope that someone may find opportunity to work out the embryology of this nematode. Such a study would be valuable and would be greatly facilitated by the thin, elastic egg shells.

In the present studies fertilized eggs, which can be distinguished by their clear nuclei, were mounted under cover slips in normal saline solution and studied under low and high powers of the microscope, a few drops of distilled water being added occasionally to compensate for evaporation. In other cases the live females containing eggs were so mounted, and in

this way external development was traced from the fertilized egg through hatching.

Under these conditions the early cleavage stages, as shown in figs. 2 to 5, develop somewhat rapidly, each cell division occurring in from ten to sixteen minutes. As segmentation proceeds and the bulk of the embryo increases, the thin, elastic shell expands accordingly. Eggs in an early cleavage stage (fig. 5) at 5 p. m. were in a morula stage with large blastomeres (fig. 6) at 8 p.m. Eighteen hours afterward development had proceeded to a morula with small blastomeres (fig. 7), in twenty more hours to a curved embryo (fig. 9), and six hours later to a coiled, motile embryo (fig. 11).

HATCHING

The stages represented in figs. 2-11 inclusive were observed in the uteri living worms mounted in normal salt solution under cover slips. Hatching occurred only after the eggs were ejected. Emergence from the egg was accomplished by repeated thrusts of the anterior end of the embryo against the thin shell which soon began to give way (fig. 12), finally rupturing (fig. 13) and liberating the writhing embryo.

FACTOR AFFECTING DEVELOPMENT

As noted above, eggs in nematodes which were in culture media developed somewhat rapidly, attaining the coiled, motile stage in approximately two days. But in the body of the live host uterine eggs do not appear to to develop beyond the four-cell stage, as numerous examinations have shown. However, such eggs in a dead and somewhat macerated cricket were in more advanced stages of development. Also in the voided feces of an infested cricket, eggs in a morula stage were found. Thus it appears that the failure of uterine eggs to develop beyond the four-cell stage in the living cricket is due to an inhibiting factor. This factor the writers believe to be lack of sufficient oxygen. In the higher animals it is well known that the intestines contain enormous numbers of bacteria which must take much of the free oxygen, especially in the colon and adjacent regions where anaerobic bacteria thrive. In the ileums of these crickets there were numerous bacteria. The cases of advanced development of uterine eggs of the nematodes in the macerated cricket would favor this interpretation, as the thin wall of the disintegrating ileum quickly ruptures, admitting oxygen. Likewise, eggs which developed up to hatching in the uteri of cultured nematodes would be in the presence of more oxygen than when in the ileum of the cricket. An ample supply of oxygen to eggs in the separate fecal pellets is obvious. That additional oxygen accelerates development in nematode eggs has been determined by the senior writer

who observed more rapid development in cultured (normal saline) eggs of *Ascaridia perspicillum* on the addition of 10% hydrogen peroxide (unpublished results).

THE HOST, GRYLLUS ASSIMILIS

DISTRIBUTION

In October, 1919, when the cricket examinations were at their height some adults were sent to Mr. James A. G. Rehn, who identified them as *Gryllus assimilis* Fabricius. Rehn and Hebard (1915, pp. 295, 296) regard this species as the common black field cricket of the Americas, which ranges from Canada to Argentina and from the Atlantic to the Pacific.

In the vicinity of Manhattan there are two races of this species, one maturing in August and September, the other in April and May. The fall adults lay their eggs in October, depositing them in the soil, under stones, and in other protected places. These eggs hatch the following spring according to Bruner (1886, p. 194), and the young mature in August and September. Concerning the occurrence and behavior of the spring adults, a few notes from the senior author's records for another problem are given, "About the last of May, 1915, adult crickets were found in nature mating. Several pairs of these were placed in cages containing sterilized earth, some carefully selected stems of alfalfa, and a small block of wood. Care was taken not to introduce any other animals." These records show that every second day the caged crickets were given one of the following foods: green alfalfa, fresh apple, algae, and small bits of fresh beef. Young crickets hatched in three weeks. By October the nymphs were approximately half-grown, averaging one-half the length of the adults.

From these and other observations it appears that in the vicinity of Manhattan, Kan., *G. assimilis* produces only one brood per year, but is represented by two races, the fall adults, laying their eggs in the autumn, passing the winter in this stage, hatching in the spring, and maturing in late summer or autumn; and the spring adults, depositing their eggs in the spring, hatching in early summer, passing the winter in a nymphal stage, and maturing the following spring. These respective findings are in close accord with the observations of McNeill (1891, p. 5) for *G. abbreviatus* in Illinois and of Blatchley (1901, p. 439) for *G. pennsylvanicus* in Indiana.

HABITS

Concerning the habits of the common black cricket, Blatchley (1901, p. 436) states that it is nocturnal, omnivorous and cannibalistic. The present studies indicate that these crickets in nature are largely nocturnal, but that they may stridulate, move about, and feed to some extent in the day time.

That they are omnivorous in nature is amply confirmed by these observations. Plants on which they have been seen feeding include alfalfa (*Medicago sativa*), bluegrass (*Poa pratensis*), bindweed (*Convolvulus spp.*), crabgrass (*Syntherisma sanguinale*), and Bermuda grass (*Capriola dactylon*). Decomposing plant and animal tissues appear not to be distasteful, as the crickets have been seen feeding on both. Portions of dead crickets and other arthropods have been taken in preference to wilted grass, and in a few instances the animal tissues were in an advanced stage of decomposition.

Cannibalism is of frequent occurrence among the common black crickets, but apparently they seldom attack each other in life. In ordinary captivity mortality is high; some of the captives usually survive and frequently feed upon their deceased mates. The senior writer in connection with another problem reared crickets from eggs in large life history cages, making almost daily observations for months. Crickets were often seen dying, and sooner or later others began to devour them. In a single instance, a live cricket was observed to approach a dying one, lying on its back, and begin feeding on the latter's hind femur. At no other time, either in 1915-16 or during the present studies, has one cricket been seen to feed upon another living one.

HABITATS

The wide distribution of *G. assimilis* is doubtless due in part to its omnivorous feeding habits and to its varied habitats. Among the habitats from which it has been taken are the following: at edges of side walks, in holes in the ground and chinks in walls of buildings, under old hardened ox feces, sticks, boards, logs, stones, and stone walls, and among various kinds of vegetation.

Besides proximity to food and a reasonable amount of protection the diurnal habitat of this cricket must afford an atmosphere of comparatively high humidity. In artificial rearing the mortality was exceedingly high until water was sprinkled into the cages, when the percentage of survivals markedly increased.

PROPORTION OF MALE AND FEMALE CRICKETS IN NATURE

The writers found the number of male and female crickets to be approximately equal in nature, except during late October after the breeding season is over. At this time the adult females, with their abdomens distended with eggs, far outnumbered the surviving adult males.

DISTRIBUTION OF CEPHALOBIUM MICROBIVORUM

GEOGRAPHICAL DISTRIBUTION

Examinations of black field crickets for *C. microbivorum* have been made in five states: Kansas, Massachusetts, Virginia, Michigan, and

Illinois, but to date these nematodes have been found only in Kansas and Virginia. From April to June, 1919, Dr. N. A. Cobb examined a few black field crickets at Falls Church, Va., and found *C. microbivorum* in nearly every cricket. At Manhattan, Kan., these nematodes are known to have been of common occurrence in the adult black crickets during the autumns of the last three years (1918, 1919, 1920).

DISTRIBUTION IN THE CRICKET HOSTS

A study of the data collected during the search for *C. microbivorum* in the local black field crickets reveals some interesting points in the distribution of these nematodes in their hosts. Most of the examinations of the crickets were made during the periods between September 19 and October 31, in 1919 and 1920. From Table I it is seen that the number of female crickets examined exceeds that of the males. This was due to certain collections made late in October after the breeding season and after the consequent heavy mortality of male crickets. Collections made in September included nearly equal numbers of males and females.

TABLE I. SHOWING NEMATODE INFESTATION OF ADULT CRICKETS EXAMINED BETWEEN SEPTEMBER 19 AND OCTOBER 31, 1919 AND 1920, AT MANHATTAN, KAN.

	No. Crickets Examined	No. Crickets Infested	Per cent Infested	Total No. Nematodes	Average Infestation per Cricket	Range of Infestation
Male Crickets	14	10	71.4	217	21.7	2 to 51
Female Crickets	33	30	90.9	822	27.4	3 to 91
All Crickets	47	40	85.1	1,029	25.7	2 to 91

From Table I it is seen that over eighty-five per cent of the crickets examined by the writers between September 19 and October 31 were infested with this nematode. Approximately seventy per cent of the males and ninety per cent of the females contained these parasites. Table I likewise shows that both the range and average infestation of the females exceed that of the males. That the intestine of the male cricket furnishes a suitable environment for these parasites is evident from infestations amounting to as many as fifty-one *C. microbivorum*. Consequently the explanation of these phenomena must be sought elsewhere. In October the females' bodies are usually gorged with eggs, and are larger than those of the males. Obviously, to afford this greater development, more food

would be required than for the males, thus increasing the chances of the females ingesting a larger number of nematode eggs or larvae. The large, distensible crop (fig. 1, cr) is adapted for receiving quantities of food, and the numerous fecal pellets voided daily by these females are evidences of large appetites. Thus, to the writers, the most plausible explanation of the higher percentage, average, and range of nematode infestations in the females is that the engorged females take more food, and thus, on the average, swallow more eggs or larvae.

Occasional examinations of three or four specimens of *G. assimilis* were made during June, August, and September, 1920. No specimens of *C. microbivorum* were found in any of these crickets until August 21 when one was taken from an adult female. Of six mature crickets—three males and three females—examined on this date, two of the females contained nematodes, the other infestation consisting of two immature specimens. In September the recorded infestations ranged from seven to thirty-one nematodes, and in October from seven to as many as ninety-one.

In addition to the examinations of adult crickets shown in Table I, some half-grown black crickets were dissected on November 3, 1920. In the ileum of one of these nymphs were two mature specimens of *C. microbivorum*. The significance of this observation will be discussed later.

PROPORTION OF SEXES AND PARTHENOGENESIS

The nematodes observed included noticeably more females than males, and in one case males were entirely lacking. This case will be discussed presently, but concerning the presence of more female nematodes than males, the writers have no explanation to offer. Merrill and Ford (1916, p. 127) likewise found the females more numerous in the two species of nematodes they studied.

In the case just mentioned in which males were lacking, the infestation consisted of three females each containing fertilized eggs. These females may have been fecundated by males which had already left the host, as two dead nematodes were found in the colon of a cricket immediately after killing. Or, the females in question may have been parthenogenetic as Welch and Wehrle (1918, p. 159) and others have observed in small nematodes. It is possible also that they may have been protandric hermaphrodites, but the almost constant occurrence of males in the infestations favors the view that the females were probably fecundated by males which subsequently passed from the cricket.

EFFECT OF NEMATODE ON HOST

The effect of *C. microbivorum* on the host does not appear to be serious, as apparently normal crickets often harbored thirty or more of these nematodes. On the other hand, it seems probable that so many compar-

atively large entozoa must be detrimental to the host. Flury (1912) has shown in the cases of nematodes parasitic in higher animals that they cause injury not only by taking food material and by stoppage, but that on account of their imperfect digestive system their excreta contain toxins which are absorbed by the host. Some of these injuries would be likely to occur in the infested crickets.

Life History of Cephalobium microbivorum

LIFE CYCLE

As stated elsewhere, *C. microbivorum* matures in the intestine of the field cricket and eggs normally develop to the four-cell stage. When such eggs are ejected and kept moist development proceeds to the coiled, motile embryo stage in about two days. Cricket feces voided during the night and examined the following morning contained eggs of *C. microbivorum* in a morula stage. To ascertain the probable fate of such eggs in nature, four adult female crickets were placed in a lantern globe cage over some moist, sterilized earth. They lived from four to six days, the dead ones being removed before they were attacked by their mates. Two of these crickets were subsequently found to be infested with mature female nematodes containing eggs. The earth in the cage was moistened nearly every day, but as it was kept uncovered in the laboratory, evaporation was rapid and the culture dried out several times. Ten days after the infested crickets were placed over the sterilized earth examinations of portions of the latter were made which revealed two dead larvae, slightly larger than newly hatched embryos.

These observations indicate that in nature the eggs pass from the body of the cricket in early cleavage stages, and since the diurnal habitat of the cricket must have an atmosphere of comparatively high humidity, as shown elsewhere, a fair percentage of the nematode eggs voided in the daytime would be protected by the humidity of the cricket habitat. Since in culturing, these nematode eggs hatched in each separate medium, it is logical to infer that they would hatch in the moist débris of a cricket habitat. The thin elastic shell which bursts after a few thrusts would probably not long confine the embryos. Another reason which leads the writers to think that these young nematodes pass a period free in nature is that while immature stages of *C. microbivorum* are found in the ileum of the cricket, these nematode larvae are always much larger than the newly hatched embryos. If infection were caused by the cricket's ingestion of nematode eggs, one would expect occasionally to find in the crickets young nematodes the size of newly hatched ones, but this has failed to occur in the removal of over one thousand of these nematodes. The writers made no attempt to infest crickets by giving them hatched embryos or free larvae, but Merrill and Ford (1916, p. 127) succeeded in infecting termites with free larvae of the nematode, *Diplogaster aerivora* Cobb.

The indiscriminate feeding habits of the crickets would give ample opportunity for the ingestion of larvae of *C. microbivorum*, for as stated elsewhere, they feed upon decomposing plant and animal tissues, and these substances are commonly found in the moist diurnal habitats. Once in the ileum of the cricket the young *C. microbivorum* evidently thrive, for they occur in active stages ranging from one-third to normal lengths.

SEASONAL ENDURANCE

In the light of the information available, this nematode's problem of enduring the seasons seems relatively simple. It has been noted that in the vicinity of Manhattan, Kan., two races of *G. assimilis* occur, one spending the winter in the egg stage, and the other in the nymphal stage. The finding in November of mature specimens of *C. microbivorum* in nymphal black field crickets which live over the winter here and elsewhere in protected places indicates one way in which the cold season may be endured. These nymphal crickets mature in May, and deposit their eggs early in June, thus making it possible to shelter these adult parasites until summer.[2] By this time the eggs of the other race are hatched, furnishing possible hosts for the young *C. microbivorum* liberated as eggs from the winter-enduring nymphal crickets. Protection of the larvae against sudden desiccation and high temperatures would be afforded deeper in the habitat. At any rate, the fact that the fall infestations are the heaviest indicates that this nematode's problem of enduring the summer is not a serious one.

Another possible means of *C. microbivorum* enduring the winter might be afforded by the habit of the fall adult crickets crawling into the ground and into other protected places when their life work is finished. If such infested crickets were killed by freezing and remained congealed throughout the winter, the spring season might be well advanced before maceration of the crickets' bodies proceeded far enough to admit sufficient oxygen for the development of the enclosed nematode eggs. The plausibility of this method is strengthened by the fact that many nematodes can withstand some freezing. It is possible, of course, that larvae of these nematodes pass the winter free in the soil. Further studies in connection with the culturing of *C. microbivorum* will doubtless settle this and other points in its life problems.

OTHER PARASITES OF THE CRICKET

While searching for *C. microbivorum* in the crickets certain other parasites were encountered; viz., gregarines, gordiacea larvae and dipterous larvae. In July, 1919, gregarines were present in many of the common black crickets examined at Woods Hole, Mass. At Douglas

[2] The writers made no examinations of the spring adults, but Doctor Cobb's examinations from April to June were of adults of this race in nearly all of which he found *C. microbivorum*.

Lake, Mich., these protozoan parasites were of frequent occurrence in crickets in August, 1920, and larval gordiacea were occasionally found.

Of the 106 mature or nearly mature crickets examined gregarines were found in thirty-seven per cent, and gordiacea larvae in 9 per cent of them. The crickets were taken from four localities; viz., Douglas Lake shore line about seventy-five yards wide; Burt Lake shore line approximately ten yards in width; Sedge Pool shore line about eight yards in width; and an upland pasture one and one-half miles from water.

The only gordiacea infestations occurred in the Sedge Pool locality, which was also the most favorable for gregarine infestations. This pool, which is separated from Douglas Lake by a narrow ledge, is approximately 200 feet long by 120 feet wide. It is protected by two to four foot banks and by a substantial growth of timber on three sides, leaving the east and southeast sides open to the direct rays of the sun. Most of the crickets were taken on the narrow ledge at the east side of the pool within ten to twenty feet of the protected water's edge. The infested crickets contained gordiacea larvae in later stages of development, some of them having attained the dark adult coloration. Two that escaped from a cricket in a bottle of water were readily identified as *Paragordius varius* (Leidy). This was the only species obtained by May (1919), who examined several hundred crickets from the east shore of Douglas Lake in connection with his studies on the life history of this species. The lowest percentage (30 per cent) of gregarine infestation was in the upland pasture one and one-half miles from Douglas Lake, while the highest (45 per cent) occurred at Sedge Pool. This indicates that these protozoan parasites thrive better under moister conditions.

Records for gregarine infestation of the crickets examined at Manhattan, Kan., are not complete, but of twenty crickets taken from nature between June 24 and October 21, 1920, eleven, or fifty-five per cent of them, were infested. The infestations ranged from one to as high as 517 gregarines, the average being slightly over sixty-two. Not a specimen of *Paragordius* was found here, but from two crickets examined by Herrick (1921) a few sarcophagid larvae were taken, this apparently being a new case of parasitism in *G. assimilis*.

Summary

1. In the autumns of 1918, 1919, 1920 black field crickets, in the vicinity of Manhattan, Kan., were infested with a new species of nematode which has been identified as *Cephalobium microbivorum* Cobb.

2. In the body of the living cricket development of the eggs has not been observed to exceed the four-cell stage. This is attributed to lack of sufficient oxygen.

3. In culturing, eggs hatched in all moist media used, but young nematodes grew only when 0.8% peptone was added, two specimens increasing their lengths 16⅔ per cent in thirteen days. In early cleavage, each cell division was accomplished in from ten to sixteen minutes; and in approximately two days the embryo was fully formed. Hatching is accomplished by repeated thrusts of the anterior end of the embryo against the thin elastic shell which soon ruptures and liberates the embryo.

4. The parasitic habitat of this nematode is the spacious ileum of *Gryllus assimilis* Fabricius, which is the common black field cricket of the Americas, ranging from Canada to Argentina and from the Atlantic to the Pacific. In the vicinity of Manhattan, Kan., this species is represented by two races, one maturing in August and September, the other in April and May; each race produces one brood of crickets per year. The race maturing in the spring winters here in the nymphal stage, while the adult fall race spends the cold season in the egg stage. Eggs of *G. assimilis* hatched (June) in three weeks after deposition. Late in October they were half grown, and by the last of the following May they were mature and mating.

5. These crickets are omnivorous, feeding on various kinds of plant and animal tissues, both fresh and decomposed. Cannibalism is of common occurrence among them. They are known to devour their dead and dying, but not to attack each other in normal condition. Their diurnal habitats, which may include a variety of situations. must furnish some protection and sustain a certain amount of moisture.

6. The numbers of adult male and female crickets observed in nature were about equal, except in the autumn after the breeding season, when more females survived.

7. Of crickets examined in five states, only those from Kansas and Virginia have been infested with *C. microbivorum*. At Manhattan, Kan., about 85 per cent of the fall adult crickets examined were infested; 70 per cent of the males and 90 per cent of the females. The females also contained a larger number of these parasites. Both the higher percentage of parasitism and the heavier infestations of the females are attributed to their greater voracity.

8. Infestations of fall adults were first found in August, the parasites being young and few. By September adult nematodes were taken and the size of infestations increased to 31. In October both young and adults were numerous, a maximum infestation amounting to 91 of these nematodes.

9. Nymphal, black field crickets of the race which winters in this stage were infested with mature nematodes in November.

10. Female nematodes were more numerous than males; for this phenomenon no explanation is offered. One cricket contained 3 female nematodes, each having fertilized eggs. Death of the males after fecundation is deemed more probable than parthenogenesis or protandric hermaphroditism.

11. No positive deleterious effect of the parasites on the host was observed, but this does not preclude possible injury.

12. The life cycle of *C. microbivorum* appears to be as follows: The nematode matures in the ileum of the common field cricket. Its eggs are deposited in early cleavage stages and passed from the body of the cricket. Under moist conditions furnished by the diurnal habitat the eggs soon hatch, and in the presence of nutritive substances the liberated embryos grow. Sooner or later the larval nematodes are swallowed by the omnivorous cricket in whose ileum they mature.

13. The nematode's problem of enduring the seasons is apparently solved by the occurrence of the two races of *G. assimilis*, the winter nymphs sheltering some of the mature nematodes through the colder months and the young of the fall adults ingesting larval nematodes during the warmer ones.

This method is probably supplemented by the infested bodies of certain fall adult crickets which, though dead, pass the winter in a somewhat congealed condition, the macerating bodies later liberating mature nematodes and eggs.

14. Other parasites encountered in the crickets examined included gregarines, gordiacea larvae and dipterous larvae. Gregarines were found generally, *Paragordius varius* larvae only at Douglas Lake, Mich., and sarcophagid larvae only at Manhattan, Kan.

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Explanation of Plate

All drawings were made with the aid of a camera lucida and are of the same magnification, X 400. The figures show stages in the embryological development of the external form of *Cephalobium microbivorum* Cobb.

Figs. 2 to 5. Eggs in early cleavage.

Fig. 6. Morula with large blastomeres.

Fig. 7. Morula with small blastomeres.

Figs. 8, 9. Eggs containing curved embryos.

Figs. 10, 11. Eggs containing coiled embryos.

Figs. 12, 13. Eggs in process of hatching.

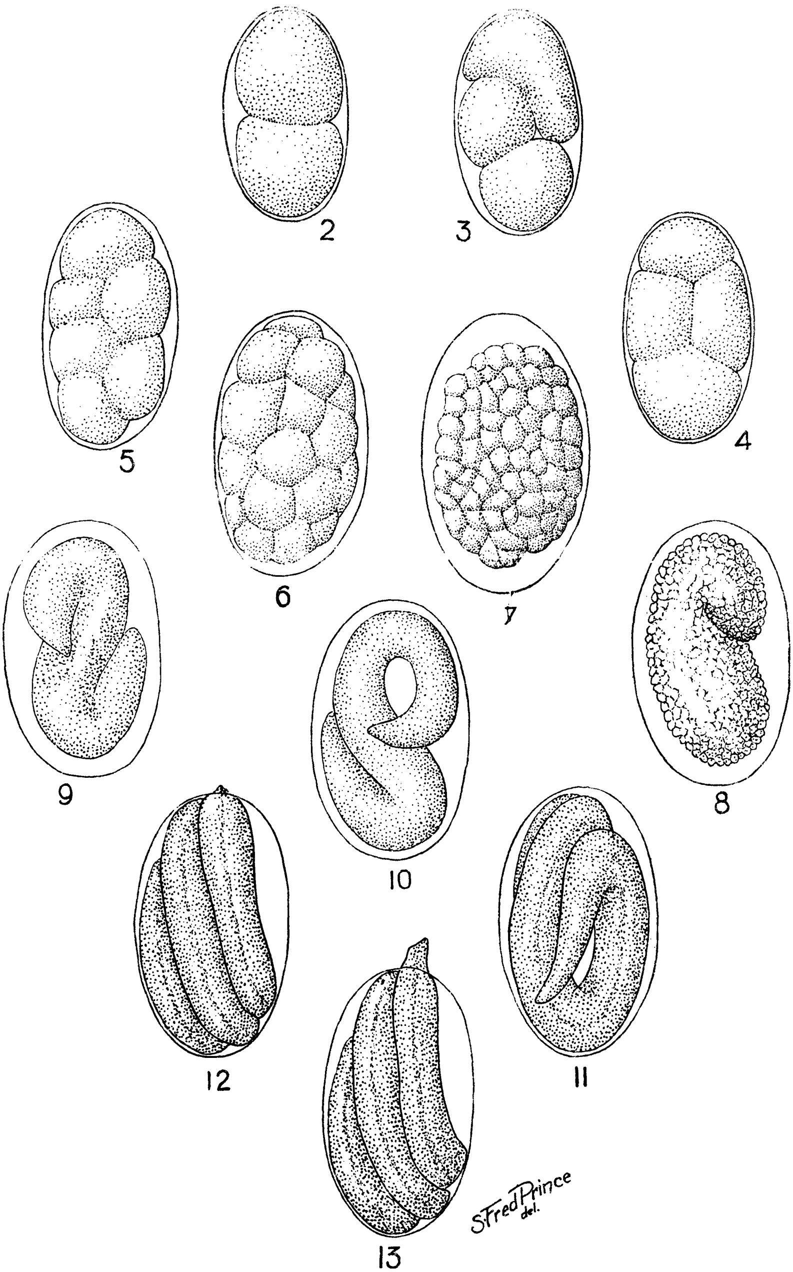

PLATE IV